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TITLE: A Novel Approach to the Development of Highly Specific Inhibitors of EFG, a Critical Transcription Factor in Prostate Cancer

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**14. ABSTRACT**

We have created a series of six constructs to express varying length fragments of ERG. Expression and purification protocols have been established for all six fragments. Using fluorescein-labeled oligonucleotides, we have developed a fluorescence polarization based assay for measuring the DNA binding of the ERG fragments to a functional DNA element. Using this assay, we have shown that longer fragments of ERG show decreased DNA binding, i.e. are auto-inhibited. Based on this data, we have focused efforts on producing one fragment in the labeled form necessary for NMR structural studies.

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prostate cancer, ERG, auto-inhibition, DNA binding, transcription factor

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## Introduction

More than 230,000 new cases of prostate cancer were diagnosed in the United States in 2006 and more than 27,000 deaths were attributed to prostate cancer, accounting for ~9% of cancer deaths in men. It is clear that there is a need for more effective highly targeted agents for the treatment of this pervasive cancer. Our goal is to explore a novel approach, namely inhibition of a specific protein, ERG, to treat this deadly disease.

We have proposed three steps to achieve our goal of finding compounds that can inhibit ERG function by stabilizing the inhibited form of the protein. First, we will make ERG in bacteria and identify which parts of the protein mediate the inhibition. Second, we will utilize nuclear magnetic resonance spectroscopy to determine the 3D structure of the inhibited form of ERG. This structural information makes it possible to use computational tools to identify molecules which may bind to ERG. Third, we will use these computational tools to identify potential lead compounds which can stabilize the inhibited form of ERG and inhibit its binding to DNA. Based on the computational results, we will test a selected panel of compounds for their ability to inhibit binding of ERG to DNA to identify compounds which are effective and can be developed further into a useful drug.

Our efforts can lay the foundation for development of drugs targeting ERG which is a novel and potentially therapeutically powerful approach for the treatment of prostate cancer.

## Body

### Aim 1: Delineation of auto-inhibitory domains of ERG.

*Generation of constructs.* Using PCR, we have generated 6 constructs in pHIS Parallel 2 thus far for expression of a series of fragments of ERG (see Table below).

| | |
|---|---|
| ERG1 | Expression of ERG residues 1-462 (full-length) |
| ERG2 | Expression of ERG residues 211-462 |
| ERG3 | Expression of ERG residues 261-412 |
| ERG4 | Expression of ERG residues 290-412 |
| ERG5 | Expression of ERG residues 290-383 |
| ERG6 | Expression of ERG residues 290-377 |

*Expression and purification.* All six of these constructs have been expressed in *E. coli* using low temperature (10 ºC) induction with IPTG. The resulting protein is expressed in a soluble form. Purification is effected using Ni-NTA affinity chromatography, rTEV cleavage of the $His_6$ tag, and a second Ni-NTA affinity chromatography step to obtain homogeneous protein.

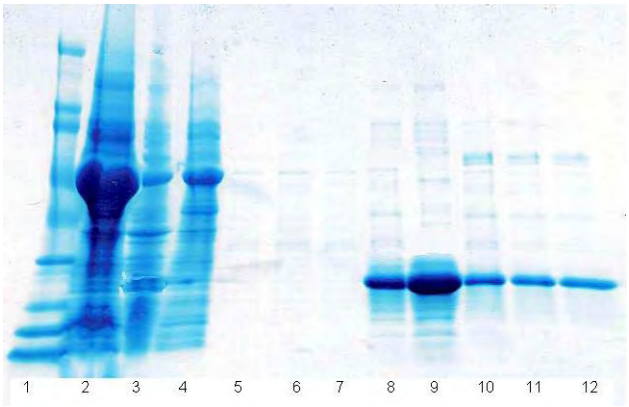


**Figure 11. SDS gel showing purification steps for $^{15}$N-labeled ERG3.** Lane 1, markers; Lane 2, flow through from Ni-NTA column; Lane 3, insoluble fraction; Lanes 4-7, wash steps on Ni-NTA column; Lanes 8-12, elution from Ni-NTA column.

*Fluorescence polarization (FP) assay for measuring ERG-DNA binding.* For the measurement of $K_d$ values for the binding of ERG fragments to DNA, we have developed a fluorescence polarization assay. Several functional DNA sequences identified from selected and amplified binding (SAAB) using the highly homologous Fli-1 protein (Mao et al., 1994) have been synthesized with a 3'-fluorescein label. Upon binding of the larger ERG protein, the fluorescence polarization is altered due to the decreased rotational correlation time of the complex, providing a simple, robust assay for these measurements (Rishi et al., 2005). A representative binding plot for binding of ERG3 to DNA is shown in Figure 2 below. We are also measuring the $K_d$ values using isothermal titration calorimetry to provide an independent determination.

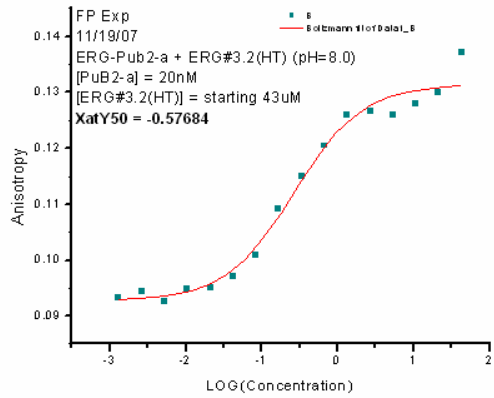


**Fig. 2. Measurement ERG3 binding to DNA using fluorescence polarization.**

This assay has been used to measure $K_d$ values for several constructs with one DNA element:

| | |
|---|---|
| ERG1 | $K_d$ = 426 nM |
| ERG3 | $K_d$ = 280 nM |
| ERG4 | $K_d$ = 120 nM |
| ERG6 | $K_d$= 100 nM |

The results of these measurements clearly that longer fragments of ERG (beyond the canonical Ets domain) do indeed show evidence of auto-inhibition, validating our original aim to explore this for ERG. The data also suggest that the ERG3 construct contains most, if not all of the regions of the protein necessary for auto-inhibition so we will focus our structural efforts on this ~150 amino acid fragment.

**Aim 2: Determination of the structure of the auto-inhibited form of ERG (X-ray or NMR).**

*Preparation of $^{15}$N-labeled protein.* ERG3 has been expressed in minimal media for labeling with $^{15}$N for preliminary NMR data collection. Good yields of labeled protein (~5 mg/L purified protein) have been obtained, establishing our ability to make the quantities of protein necessary for the NMR studies (see Figure 1 above).

**Aim 3: Virtual screening of ERG structure to identify potential lead compounds.**

We do not yet have a structure of the protein, so efforts on this aim have not yet begun.

## Key Research Accomplishments

- Creation of a serried of constructs for expression of various fragments of ERG
- Development of expression and purification protocol for fragments of ERG
- Development of fluorescence polarization (FP) assay for measurement of ERG-DNA binding
- Analysis of ERG fragment – DNA binding by FP

## Reportable Outcomes

- We plan to use the preliminary data generated under the auspices of this grant to seek additional federal funding for this project through DOD or NIH.
- One student, Tomasz K. Kabzinski, has been supported by this grant.

## Conclusion

We have confirmed that, like Ets-1, ERG shows evidence of auto-inhibition of DNA binding, laying the groundwork for our subsequent studies. Based on the results, we have focused our structural efforts on one ~150 amino acid fragment of ERG, ERG3.

## References

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